JC13 Rec'd PCT/PTO 06 APR 2001

FORM-PTO-1390 (Rev. 12-29-99) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: 004080-164

U.S. APPLICATION NO. (If known, see 37 C.F.R. 1.5): 09/807013

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/SE99/01788	6 October 1999	6 October 1998

TITLE OF INVENTION: USE OF SOCS-2 OR CIS TO SCREEN FOR COMPOUNDS ENHANCING GROWTH HORMONE EFFECT

APPLICANT(S) FOR DO/EO/US: Gunnar NORSTEDT, Petra TOLLET EGNELL, and Amilcar Flores MORALES

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and the PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US)
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☒ Other items or information: Copy of International Search Report, and copy of International Preliminary Examination Report
    International Preliminary Examination Report

JC02 Rec'd PCT/PTO 06 APR 2001

U.S. APPLICATION NO. (If known, see 37 C.F.R. 1.5) 09/807013	INTERNATIONAL APPLICATION NO. PCT/SE99/01788	ATTORNEY'S DOCKET NUMBER 004080-164

17. ☒ The following fees are submitted:	CALCULATIONS	PTO USE ONLY
Basic National Fee (37 CFR 1.492(a)(1)-(5)):		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO $1,000.00 (960)		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO $860.00 (970)		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO $710.00 (958)		
International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) $690.00 (956)		
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) $100.00 (962)		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 860.00	
Surcharge of **$130.00 (154)** for furnishing the oath or declaration later than 20 ☐ 30 ☒ months from the earliest claimed priority date (37 CFR 1.492(e)).	$ 130.00	

Claims	Number Filed	Number Extra	Rate		
Total Claims	10 -20 =	0	X$18.00 (966)	$ 0.00	
Independent Claims	2 -3 =	0	X$80.00 (964)	$ 0.00	
Multiple dependent claim(s) (if applicable)			+$270.00 (968)	$	
TOTAL OF ABOVE CALCULATIONS =				$ 990.00	
Reduction for 1/2 for filing by small entity, if applicable (see below).				$ 495.00	-
SUBTOTAL =				$ 495.00	
Processing fee of **$130.00 (156)** for furnishing the English translation later than 20 ☐ 30 ☐ months from the earliest claimed priority date (37 CFR 1.492(f)). +				$	
TOTAL NATIONAL FEE =				$ 495.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00 (581)** per property +				$	
TOTAL FEES ENCLOSED =				$ 495.00	
				Amount to be: refunded	$
				charged	$

a. ☒ Small entity status is hereby claimed.

b. ☒ A check in the amount of $ 495.00 to cover the above fees is enclosed.

c. ☐ Please charge my Deposit Account No. 02-4800 in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.

d. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 02-4800. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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39,300
REGISTRATION NUMBER

09/807013
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Patent
Attorney's Docket No. 004080-164

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of	)	
	)	
Gunnar NORSTEDT et al	)	Group Art Unit: Unassigned
	)	
Application No.: TBA (§371 of PCT/SE99/01788)	)	Examiner: Unassigned
	)	
Filed: Herewith	)	
	)	
For: USE OF SOCS-2 OR CIS TO SCREEN FOR COMPOUNDS ENHANCING GROWTH HORMONE EFFECT	)	

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to examination of the above-captioned patent application, kindly enter the following amendment.

IN THE CLAIMS:

Kindly replace claim 9 as follows.

9. (Amended) A method according to claim 7 that reduces the biological activity of SOCS-2 and/or CIS comprising purified or recombinant SOCS-2 and/or CIS and proteins, optionally isolated, that link SOCS-2 and/or CIS to signaling molecules dependent on GH.

Kindly replace claim 10 as follows.

10. (Amended) A method according to claim 7 to increase cellular sensitivity to GH characterized by a reduction of levels of SOCS-2 and/or CIS.

REMARKS

By the present Amendment, claim 9 has been amended to correct a minor typographical error. Claim 10 has been amended to correct multiple dependency. No new matter has been added.

In the event that there are any questions concerning this amendment, or the application in general, the Examiner is respectfully urged to telephone the undersigned so that prosecution of the application may be expedited. Favorable consideration on the merits is respectfully requested.

Respectfully submitted,

BURNS, DOANE, SWECKER & MATHIS, L.L.P.

By: _____
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Date: 6 April 2001

Attachment to PRELIMINARY AMENDMENT dated April 6, 2001

Marked-up Claims 9 and 10

9. (Amended) A method according to claim 7 that [reduce] reduces the biological activity of SOCS-2 and/or CIS comprising purified or recombinant SOCS-2 and/or CIS and proteins, optionally isolated, that link SOCS-2 and/or CIS to signaling molecules dependent on GH.

10. (Amended) A method according to claim 7[-9] to increase cellular sensitivity to GH characterized [characterised] by a reduction of levels of SOCS-2 and/or CIS.

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USE OF SOCS-2 OR CIS TO SCREEN FOR COMPOUNDS ENHANCING GROWTH HORMONE EFFECT

Field of the invention

This invention relates to the identification of a cellular mechanism that determines the sensitivity towards a hormonal signal, and a strategy to interfere with the above mentioned cellular mechanism in order to pharmacologically change hormonal sensitivity. More specifically, this invention relates to the provision of a method to increase the cellular sensitivity to growth hormone (GH) by the interference of a so-called SOCS (suppressor of cytokine signaling) signal. Said provision is obtained by the use of SOCS-2 or CIS to screen for compounds which enhance the effects of grow hormone.

Background of the invention

GH is a hormone that is widely used as a therapeutical agent. The most well known medical indication for GH treatment is dwarfism, however, other medical areas where GH treatment might be indicated have been suggested. These areas include certain lipid disorders, heart conditions and disorders of body composition, metabolism and ageing. The presently used GH therapy normally consists of daily injections of a relatively large (198 amino acid) recombinantly manufactured GH. This type of treatment is associated with a relatively high cost and inconvenience for the patient. In light of the expected increase of the medical use for GH, compounds has been searched for that would serve as substitutes for GH. Indeed, such attempts have resulted in the discovery of compounds that increase the pituitary GH production. Another theoretical alternative would be to manufacture a compound that would mimic the cellular mechanism of GH action. In this regard it is relevant to note that the cellular mechanism of GH action is nowadays partly understood (1). A primary step involves binding of GH to its membrane bound receptor, after which receptor homodimerisation occurs. In turn this leads to the activation of the receptor associated kinase JAK2 (janus kinase 2). Once activated, JAK2 is able of stimulating other downstream pathways such as STAT (signal transducer and activator of transcription), MAPK (mitogen activated protein kinase) and IRS-1/PI3 kinase (insulin receptor substrate 1/phosphatidylinositol 3 kinase). GH activation of the JAK2 pathway is transient in nature; e.g. GH activated phosphorylated JAK2, as well as its downstream target STAT5, are only present during approximately 30 minutes in cells stimulated by GH. Interestingly, the duration of the time STATs are activated

can be prolonged by the addition of blockers of protein synthesis (2). This supports the concept that proteins might exist that have the capacity to "shut down" the GH receptor. In another research area that involves the action of cytokines such as interferons and interleukins, a new class of proteins, called SOCS (Suppressors Of Cytokine Signaling) (3, 4) were recently discovered. The SOCS proteins are made in response to cytokine stimulation and block then the activated receptor. At least seven different SOCS proteins have been characterized, and computer-based searches have identified more that 30 different proteins that could contain a SOCS motif in their amino acid sequence. A common feature of these well-defined SOCS proteins is the presence of one SH2 domain (Src homology domain 2) and a novel type of SOCS motif. As stated above, the SOCS were discovered when studying the actions of interleukins and interferons. The ability of certain SOCS proteins to block signaling from interferone/interleukin receptors has been shown in experiments using transfection of cells. In a recent study (5), SOCS-3 was reported to be regulated by GH in cultured cells. To a certain extent this finding was anticipated, since the GH receptor is a member of the cytokine receptor family. The recent discovery of the SOCS family of proteins, taken together with their function as proteins that can "turn off" cytokine receptors, is likely to have led many scientists into a contemplation of their potential use as drug targets. However, as stated above, the fact was that many different SOCS exist and the problem to be solved was to find out which of the different SOCS should be used as a drug target in order to influence a specific function of a cytokine.

In the prior art it is also a well-established clinical and experimental finding that GH deficient subjects are extremely sensitive to GH. Upon prolonged GH treatment this extreme GH sensitivity is reduced.

In the prior art it has been shown that GH regulates SOCS expression, Adams TE et al, "Growth Hormone Preferentially Induces the Rapid, Transient Expression of SOCS-3, a novel inhibitor of cytokine receptor signaling", 1998, vol 273(3), p. 1285ff. This publication contains no information of SOCS levels in GH hypersensitive states. The choice of model cell systems and the particular design of an animal experiment in this publication does not adequately approach the question of hypersensitivity towards GH. This explains that the

conclusions drawn are somewhat different in the work of Adams et al compared to what is the basis for the present invention.

In WO 98/20023 different SOCS sequences have been described as is the use of SOCS to modify cytokine functions; little reference is given to GH in this application and there is no reference to individual SOCS and states of GH sensitivity.

The present invention

We have found that the mechanism which causes this high GH sensitivity is a useful target for drug interference. Such a drug would cause the cell to become more sensitive to an endogenous GH signal. Based on a substantial research effort in the area of GH signaling, we have to our surprise found two molecular targets that can determine GH sensitivity. These targets, defined as SOCS-2 and CIS (cytokine induced SH2-containing protein), have been found to be selectively reduced in conditions of GH deficiency. We have also found that these SOCS proteins are GH regulated. The GH deficient state itself does not represent a severe medical threat to an individual, which leads to the realisation that SOCS-2 and CIS can be reduced in an organism without any severe medical consequences.

The principle underlying the present invention is that a reduction of SOCS-2 and/or CIS will increase the cellular sensitivity to GH. The finding that the above mentioned SOCS are reduced in a situation only associated with growth failure, and not with other severe malfunction of the body, indicates a certain specificity for GH in the SOCS-2/CIS system. In turn, this suggests that SOCS-2 and/or CIS could be useful targets to find GH sensitising agents. In the present invention, a cell system is described that could be used to screen for inhibitors of SOCS-2 and CIS. Alternative screening strategies are also exemplified. A therapeutical use of compounds that reduce the levels of SOCS-2 and/or CIS would lead to an increased sensitivity to GH. Such a compound might find its use in patients that suffer from syndromes of GH resistance or insufficient levels of GH. The terminology; reduction of the level of SOCS-2 and/or CIS, refers to a reduction of cellular SOCS-2 and/or CIS protein content either by an interference with (i) the synthetic machinery e.g. by reduction of the corresponding SOCS mRNAs or (ii) mechanisms that regulate protein turn-over. In addition

reduction of SOCS-2 and CIS could also refer to a reduction of biological activity of SOCS-2 and CIS by e.g. an interference with key functional domains in the proteins.

Description of the invention

The invention relates to the use of SOCS-2 and/or CIS to screen for compounds which enhance the effects of growth hormone. SOCS-2 and/or CIS, according to the invention, are used as targets in the screening of GH sensitisers. Such a sensitiser, is a compound that either reduces the cellular content of SOCS-2 and/or CIS, or serve as a blocker of the interaction of SOCS-2 and/or CIS with key signaling components of the GH transduction pathway.

The invention also relates to the monitoring of SOCS-2 and/or CIS and further includes the use of SOCS-2 and/or CIS in a conventional assay system to screen for compounds that reduce SOCS-2 and/or CIS comprising cultured cells in which levels (protein and/or mRNA) of SOCS-2 and/or CIS can be monitored. The invention also comprises the use of an assay system to screen for compounds that reduce the biological activity of SOCS-2 and/or CIS comprising purified or recombinant SOCS-2 and/or CIS and proteins, optionally isolated, that link SOCS-2 and/or CIS to signaling molecules dependent on GH.

A further embodiment relates to a method of screening for compounds that reduces SOCS-2 and/or CIS comprising cultured cells in which levels of SOCS-2 and/or CIS can be monitored and yet another to a method to screen for compounds that reduce the biological activity of SOCS-2 and/or CIS comprising purified or recombinant SOCS-2 and/or CIS, and proteins, optionally isolated, that link SOCS-2 and/or CIS to signaling molecules dependent on GH. The invention also comprises a method to increase cellular sensitivity to GH by reduction of the levels of SOCS-2 and/or CIS.

We have identified that SOCS-2 and/or CIS serve as useful targets to find GH sensitisers. We claim that a compound, that would either reduce the cellular content of SOCS-2 and/or CIS, or serve as a blocker of the interaction of SOCS-2 and/or CIS with key signaling components of the GH transduction pathway, will sensitise for GH. In the present invention we also provide strategies to find such compounds.

Materials and methods as used according to the invention are in line with established techniques. The invention encompasses all embodiments as disclosed in the claims.

Examples of the invention

The examples as provided demonstrate that a cellular reduction in SOCS-2 and CIS levels is associated with a GH hypersensitive state, and that GH regulates the expression of SOCS-2 as well as CIS. An example is also provided of a cell system that according to which compounds can be found that reduce the functions of SOCS-2 as well as CIS. Finally, we outline a strategy to design an in vitro assay to identify a compound that inhibits the interaction of SOCS-2 as well as CIS with signaling components of the GH transduction pathway. These examples are used only to illustrate the invention and are not intended to limit the scope of the invention.

Example 1

A GH hypersensitive state is associated with a reduction of SOCS-2 mRNA and CIS mRNA

Chronically (2 weeks) adult hypophysectomized (HX) male and female rats were compared to intact controls. A selection of different tissues (all known to be GH responsive) consisted of liver, muscle and adipose tissue. From these tissues RNA were prepared and levels of SOCS-2 mRNA, CIS mRNA and SOCS-3 mRNA were analysed using a RNAse protection/solution hybridisation assay (6). The probes that were used to detect these transcripts were isolated from rat genomic DNA using PCR technique with the following primers:

SOCS-3 Forward primer: 5' GAG TAC CCC CAA GAG AGC TTA CTA C 3'
Reverse Primer: 5' CTC CTT AAA GTG GAG CAT CAT ACT G 3'
PCR product length: 209 bases

SOCS-2 Forward Primer: 5' GAG CTC AGT CAA ACA GGA TGG TAC T 3'
Reverse Primer: 5' AGA ATC CAA TCT GAA TTT CCC ATC T 3'
PCR product length: 201 bases

CIS: Forward Primer: 5' ATC TTG TCC TTT GCT GGC TGT 3'
Reverse Primer: 5' CCC GAA GGT AGG AGA ACG TCT 3'
PCR product length: 215 bases

The PCR products were cloned into a plasmid vector containing T3/T7 promoter.
In this experiment, hypophysectomy caused the expected growth failure, but in other aspects the animals were in good condition. As can be seen in Fig 1, hypophysectomy caused a dramatic reduction in transcript levels of SOCS-2 and CIS, whereas the expression of SOCS-3 was not altered. This was the situation in liver (Fig 1A), muscle (Fig1B) and fat (Fig1C). The marked reduction of these two SOCS transcripts, taken together with the previously established SOCS function (to block cytokine receptors such as the GH receptor) and the well known state of GH increased GH sensitivity in hypophysectomised rats, allows one to draw the conclusion that SOCS-2 and/or CIS could be a major determinant of GH sensitivity in different tissues.

Example 2

GH regulates the expression of SOCS-2

In a follow up experiment, GH (hGH 5 ug/h/250 mg BW) was infused into hypophysectomised (HX) rats for one week using osmotic minipumps. The experiment also included a group of intact female rats and a group of HX rats, each group consisted of four animals and SOCS-2 mRNA was analysed in RNA prepared from liver. As shown in Fig 2, GH treatment caused a partial restoration of the SOCS-2 mRNA level, indicating that SOCS-2 transcripts are GH regulated. These data fit well with a concept of SOCS proteins being GH receptor "switch off" signals, and the known situation in which a continued GH treatment reduce GH sensitivity.

Example 3

A cell system to be used to interfere with functions of SOCS-2 and CIS

Rat liver hepatocytes were isolated and cultured on matrigel in serum free Williams E medium. Hormonal treatments were started 40 hours after seeding. In a time course study, SOCS-2, SOCS-3 and CIS mRNA were measured and the results are shown in Fig 3. From the data it is clear that all of the different SOCS transcripts were increase by GH. The kinetics were however different; SOCS-3 was only transiently increased by GH, whereas SOCS-2 and CIS mRNA responded to GH treatment by a continued increase for the duration of the experiment. Taken together with the results from experiment 1, these data imply that SOCS-2

and CIS are the prime candidates to determine GH sensitivity. It is also demonstrated that SOCS-2 and CIS were detectable in a situation without GH stimulus. For this reason it would be possible to use unstimulated cells in a drug screening experiment to search for compounds that reduce the levels of SOCS-2 and/or CIS. In such an experiment an assay could be based on the detection of SOCS-2 mRNA and/or CIS mRNA using conventional means (RNA/RNA hybrid formations or DNA/RNA hybrid formations) for mRNA detection. Alternatively, the SOCS-2 and/or CIS protein could be detected by immunological techniques or by genetically engineer a cell to express a tagged SOCS-2/CIS protein.

It should also to be noted that other types of tissues, such as muscle and adipose tissue, responded in a similar manner as liver to GH deficiency and treatment (Fig 1B and C). Subsequently, it should likewise be possible to develop a screening assay using cells derived from muscle or adipose tissue. Alternatively, any cell-line that express GH receptors and SOCS-2 and/or CIS could be used. In yet another embodiment a screening strategy could be designed in cells that uses the combination of GH addition and SOCS-2/CIS reduction. The end point measuring parameters would then measure both facilitation of a GH signal, in the form of e.g. a proliferative response, a metabolic alteration or a reporter gene activation in combination with the above mentioned way to measure a SOCS-2/CIS reduction.

Example 4

In vitro assays to identify a compound that inhibits the interaction of SOCS-2 and/or CIS with signaling components of the GH signal transduction pathway.

SOCS-2 and/or CIS is likely to physically bind to an activated GH receptor-JAK2 complex, either in a direct manner or via an as yet unknown bridging molecule. An activated GH receptor-JAK2 complex can be obtained in several different ways; by immunoprecipitation of membrane extracts from normal cells or tissues, or from cells that have been genetically engineered to express the GH receptor-JAK2 complex. Alternatively, relevant parts of the GH receptor and the JAK2 proteins can be recombinantly expressed and purified. An in vitro assay would then be designed to first establish an assay that would detect the binding between the GH receptor-JAK complex and the preferably recombinantly expressed SOCS-2/CIS protein. For this purpose an easy detection system should be available for at least one of the partners in the complex; such a detection system could be based on antibodies, or on

"tagging" one of the proteins with a marker molecule or radioactivity. The subsequent use of this assay would be to screen a compound-collection for substances that would block the interaction between the GH receptor-JAK2 complex and SOCS-2/CIS.

References:

1.Wood TJ, Haldosén L-A, Sliva D, Sundström M and Norstedt G Stimulation of kinase cascades by growth hormone; a paradigm for cytokine signaling. In; Progress in Nucleic Acid Research and Molecular Biology, 57: 73-94 (1997)

2. Fernandez L, Flores-Morales A, Lahuna O, Sliva D, Norstedt G, Haldosén L-A, Mode A, and Gustafsson J-Å Desenitization of the growth hormone-induced Janus kinase 2 (JAK2)/signal transducer and activator of transcription 5 (Stat5)-signaling pathway requires protein synthesis and phospholipase C. Endocrinology 139: 1815-1824 (1998)

3. Starr R, Willson T A, Viney E M, Murray L J L, Rayner J R, Jenkins B J, Gonda T J, Alexander W S, Metcalf D, Nicola N A and Hilton D J A family of cytokine-inducible inhibitors of signaling Nature, 387: 917-921 (1997)

4. Endo T A, Masuhara M, Yokouchi M, Suzuki R, Sakamoto H, Mitsui K, Matsumoto A, Tanimura S, Ohtsubo M, Misawa H, Miyazaki T, Leonor N, Taniguchi T, Fujita T, Kanakura Y, Komiya S and Yoshimura A A new protein containing SH2 domain that inhibits JAK kinases Nature, 387: 921-924 (1997)

5. Adams T E, Hansen J A, Starr R, Nicola N A, Hilton D J, and Billestrup N Growth hormone preferentially induces the rapid, transient expression of SOCS-3, a novel inhibitor of cytokine receptor signaling J Biol Chem 273: 1285-1287 (1998)

6. Möller C, Arner P, Sonnenfeld T, Norstedt G: Quantitative comparision of insulin-like growth factor I (IGF-I) and IGF-II messenger RNA levels in human and rat tissues analysed by a solution hybridization assay. J. Molecular Endocrinology, 7:213, 1991.

Claims

1. The use of SOCS-2 and/or CIS to screen for compounds that reduce the cellular expression of SOCS-2 and/or CIS and thereby enhance the effects of growth hormone.

2. The use according to claim 1 of SOCS-2 in purified or recombinant form.

3. The use according to claim 1 of CIS in purified or recombinant form.

4. The use according to claim 1 of SOCS-2 and/or CIS in a conventional screening assay system.

5. The use according to claim 4 in an assay system to screen for compounds that reduce SOCS-2 and/or CIS comprising cultured cells in which levels of SOCS-2 and CIS can be monitored.

6. The use according to claim 4 in an assay system to screen for compounds that reduce the biological activity of SOCS-2 and/or CIS comprising purified or recombinant SOCS-2 and/or CIS and proteins, optionally isolated, that link SOCS-2 and/or CIS to signaling molecules dependent on GH.

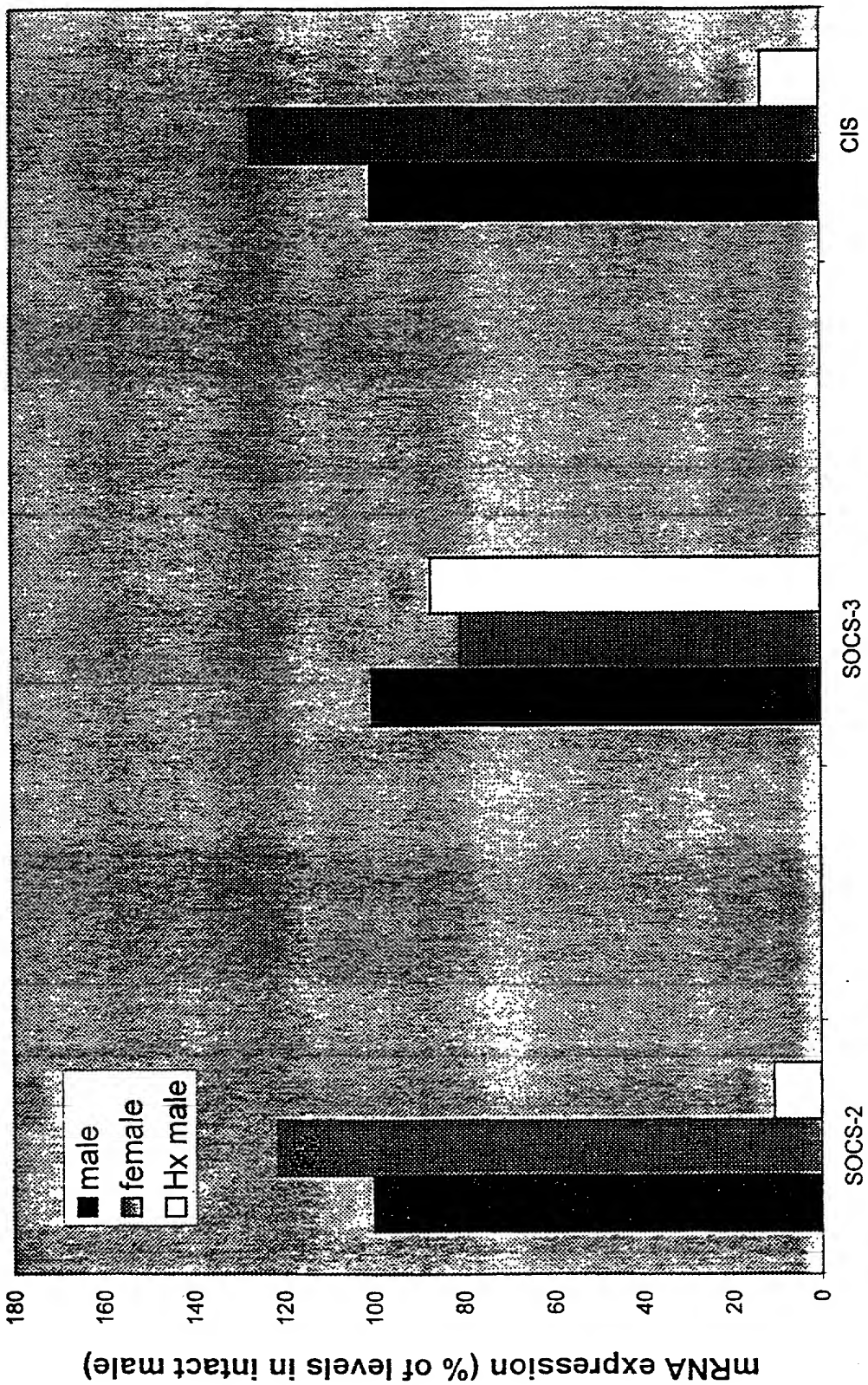
7. A method to screen for compounds that reduce SOCS-2 and/or CIS and thereby enhance the effects of growth hormone.

8. A method according to claim 7 comprising cultured cells in which levels of SOCS-2 and/or CIS can be monitored.

9. A method according to claim 7 that reduce the biological activity of SOCS-2 and/or CIS comprising purified or recombinant SOCS-2 and/or CIS and proteins, optionally isolated, that link SOCS-2 and/or CIS to signaling molecules dependent on GH.

10. A method according to claims 7 - 9 to increase cellular sensitivity to GH characterised by a reduction of levels of SOCS-2 and/or CIS.

Fig 1A, Liver


mRNA expression (% of levels in intact male)
180
160
140
120
100
80
60
40
20
0
male
female
Hx male
SOCS-2
SOCS-3
CIS

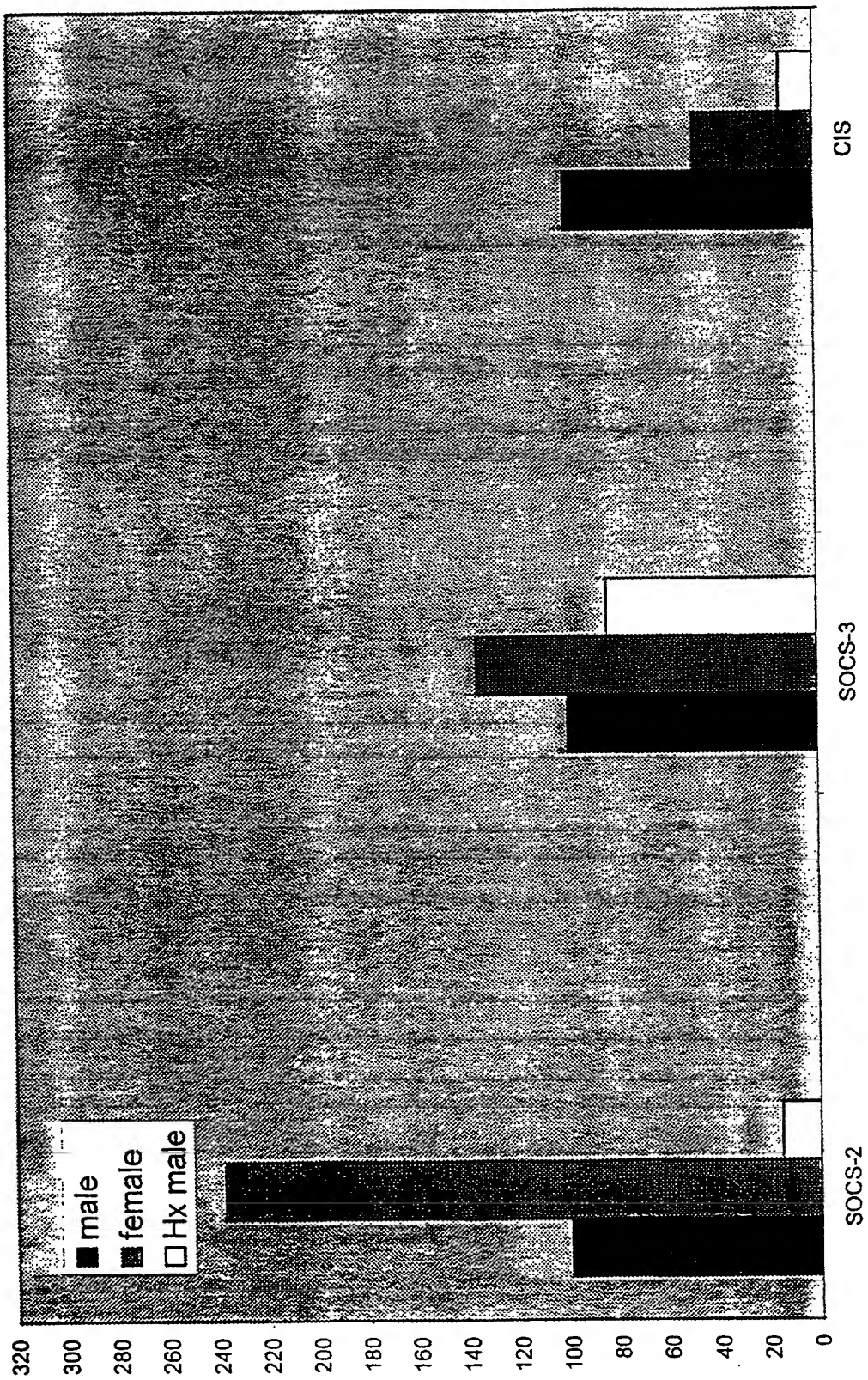


Fig 1B, Muscle

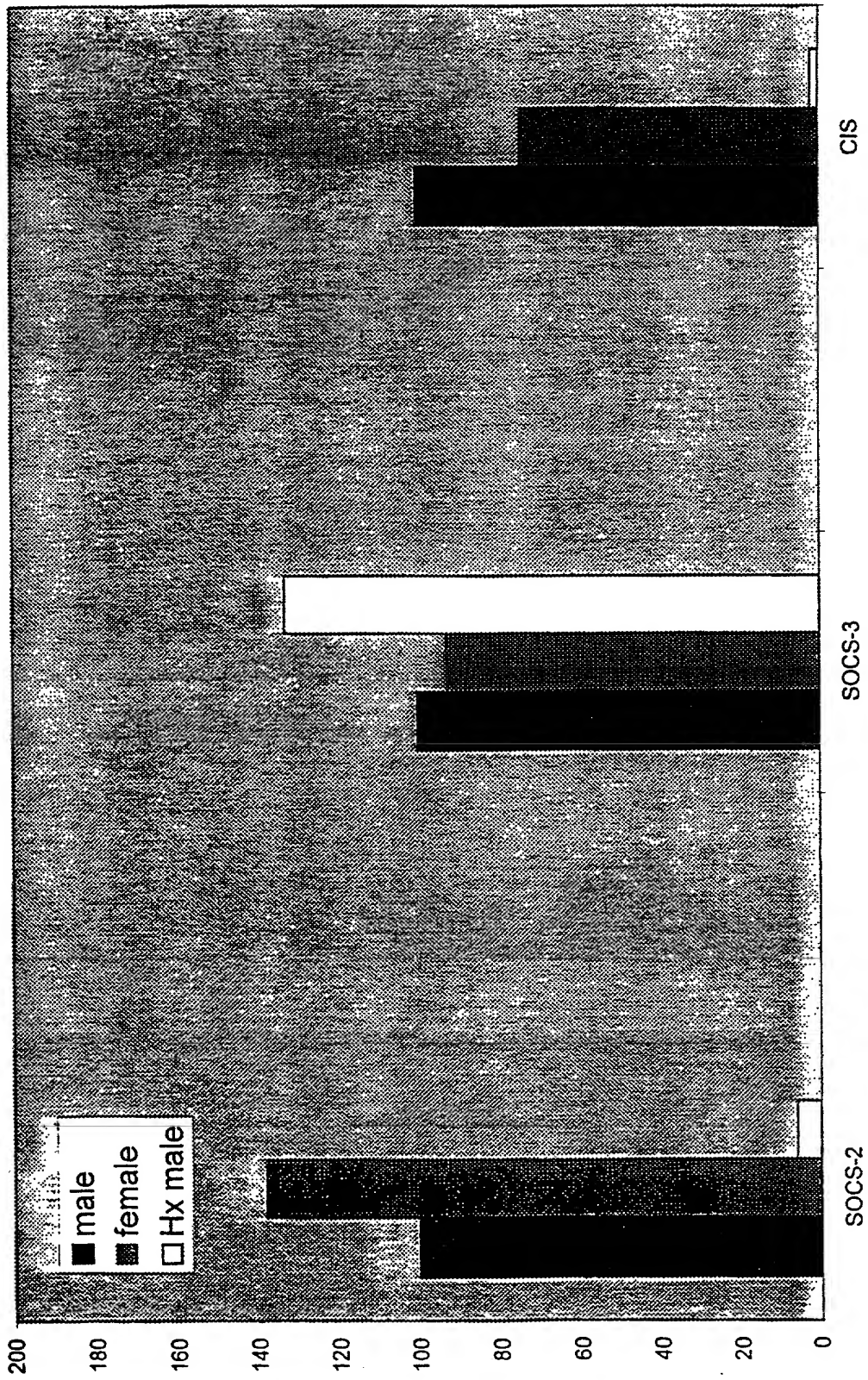
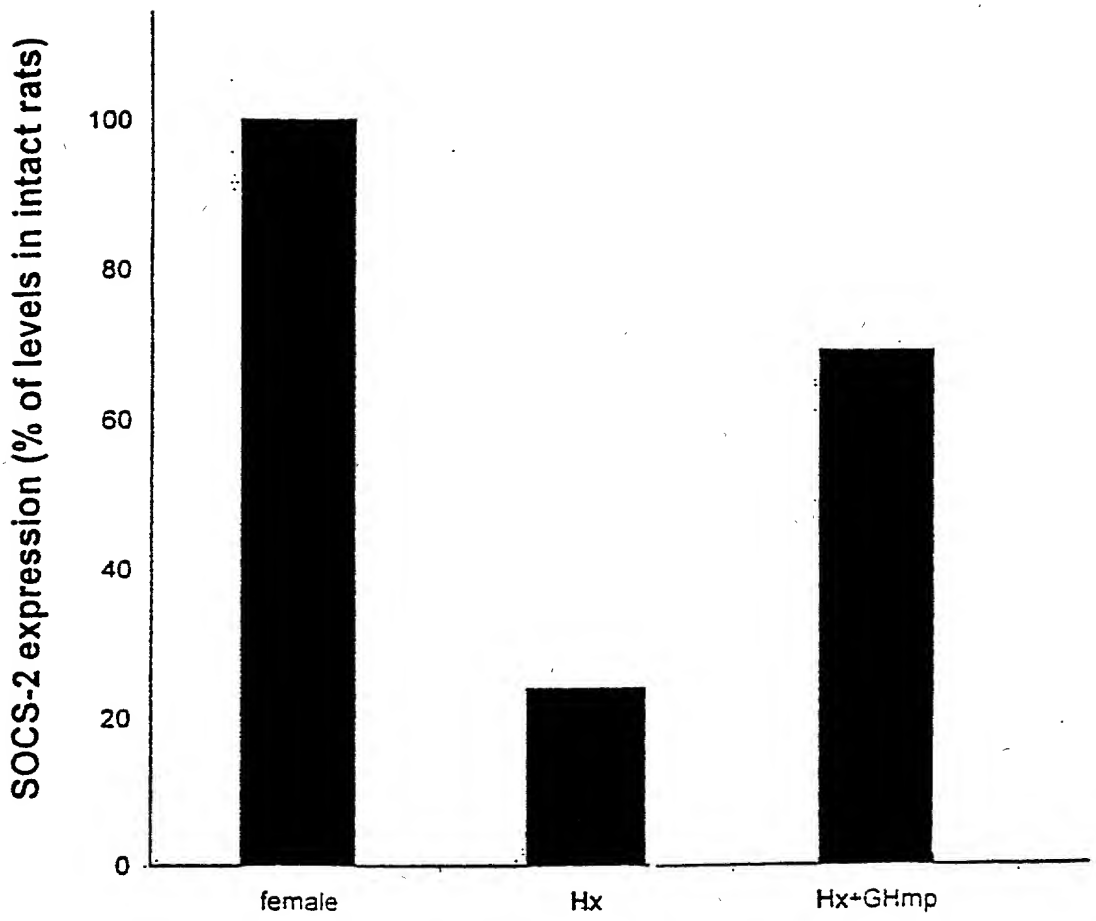


Fig 1C, Adipose tissue

Fig 2, Liver


SOCS-2 expression (% of levels in intact rats)
100
80
60
40
20
0
female
Hx
Hx+GHmp

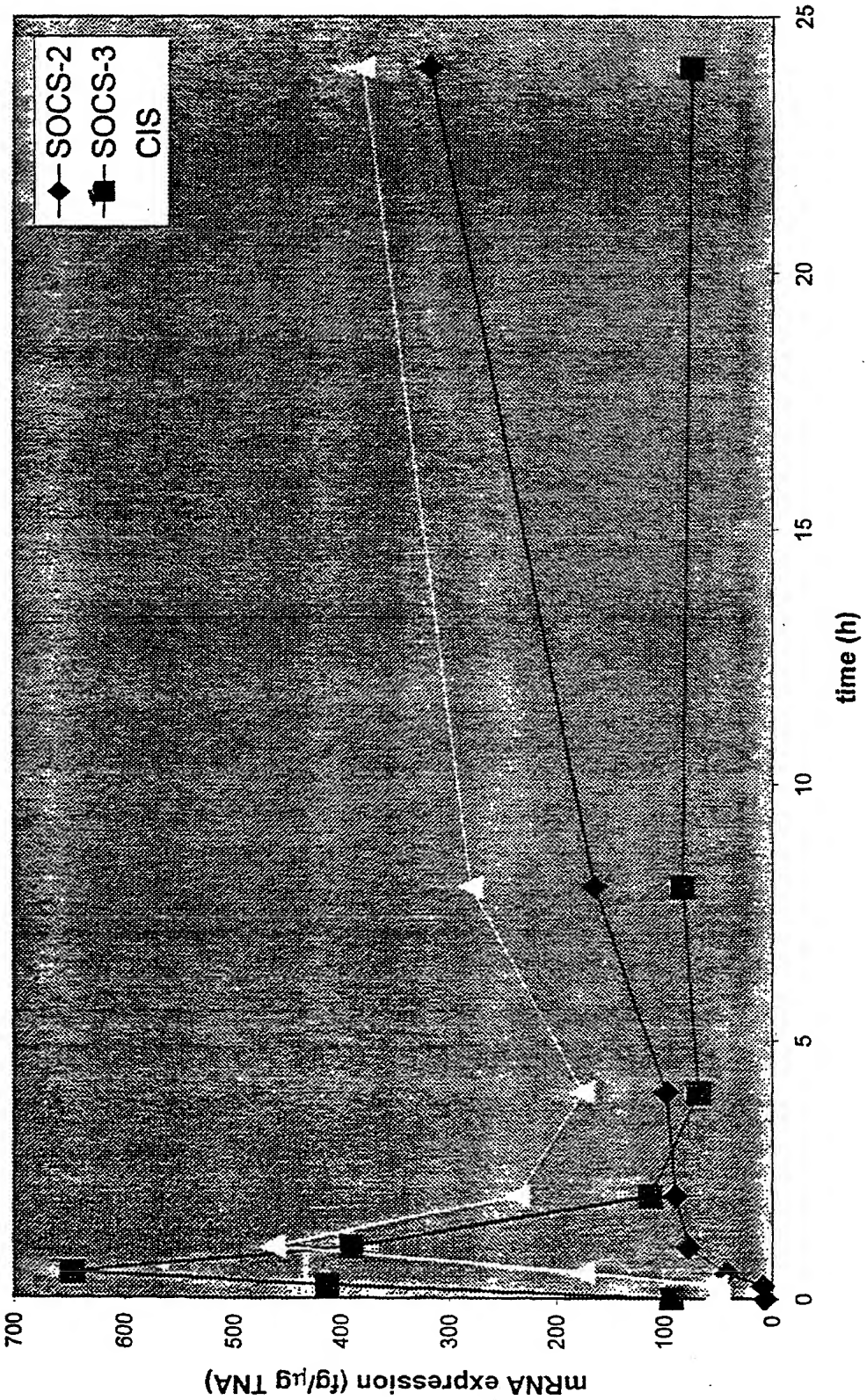


Fig 3, Hepatocytes

COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY
(Includes Reference to Provisional and PCT International Applications)

Attorney's Docket No.
004080-164

As a below named inventor, I hereby declare that:
My residence, post office address and citizenship are as stated below next to my name;
I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

USE OF SOCS-2 OR CIS TO SCREEN FOR COMPOUNDS ENHANCING GROWTH HORMONE EFFECT

the specification of which (check only one item below):

☐ is attached hereto.

☐ was filed as United States application
Number _____
on _____
and was amended
on _____ (if applicable).

☒ was filed as PCT international application
Number PCT/SE99/01788
on 6 October 1999
and was amended
on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119 (a)-(e) of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

PRIOR FOREIGN/PCT APPLICATION(S) AND ANY PRIORITY CLAIMS UNDER 35 U.S.C. §119:

COUNTRY (if PCT, indicate "PCT")	APPLICATION NUMBER	DATE OF FILING (day, month, year)	PRIORITY CLAIMED UNDER 35 U.S.C. §119
PCT	SE99/01788	6 October 1999	X Yes _ No
Sweden	9803398-8	6 October 1998	X Yes _ No
			_ Yes _ No
			_ Yes _ No
			_ Yes _ No

I hereby claim the benefit under Title 35, United States Code § 119(e) of any United States provisional application(s) listed below.

_____ (Application Number) _____ (Filing Date)

_____ (Application Number) _____ (Filing Date)

(01/01)

COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY (CONT'D) (Includes Reference to Provisional and PCT International Applications)	Attorney's Docket No. 004080-164

I hereby claim the benefit under Title 35, United States Code, §120 of any United States applications(s) or PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose to the Office all information known to me to be material to the patentability as defined in Title 37, Code of Federal Regulations §1.56, which became available between the filing date of the prior application(s) and the national or PCT international filing date of this application:

PRIOR U.S. APPLICATIONS OR PCT INTERNATIONAL APPLICATIONS DESIGNATING THE U.S. FOR BENEFIT UNDER 35 U.S.C. §120:

U.S. APPLICATIONS		STATUS (check one)		
U.S. APPLICATION NUMBER	U.S. FILING DATE	PATENTED	PENDING	ABANDONED

PCT APPLICATIONS DESIGNATING THE U.S.					
PCT APPLICATION NO.	PCT FILING DATE	U.S. APPLICATION NUMBERS ASSIGNED (if any)			

I hereby appoint the following attorneys and agent(s) to prosecute said application and to transact all business in the Patent and Trademark Office connected therewith and to file, prosecute and to transact all business in connection with international applications directed to said invention:

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY (CONT'D) (Includes Reference to Provisional and PCT International Applications)	Attorney's Docket No.

FULL NAME OF SOLE OR FIRST INVENTOR	SIGNATURE	DATE
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FULL NAME OF FIFTH JOINT INVENTOR, IF ANY	SIGNATURE	DATE
RESIDENCE	CITIZENSHIP	
POST OFFICE ADDRESS		

FULL NAME OF SIXTH JOINT INVENTOR, IF ANY	SIGNATURE	DATE
RESIDENCE	CITIZENSHIP	
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FULL NAME OF SEVENTH JOINT INVENTOR, IF ANY	SIGNATURE	DATE
RESIDENCE	CITIZENSHIP	
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FULL NAME OF EIGHTH JOINT INVENTOR, IF ANY	SIGNATURE	DATE
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